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Research Article

Effects of Gender and Age on Immunoglobulin M (IgM) Profile of Sumbawa Horses Raised in the Traditional Grazing System

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ABSTRACT

Introduction: Sumbawa horses are valued for their cultural, economic, and functional roles in Indonesia. Sumbawa horses are traditionally raised under the Lar system, where environmental and physiological factors, such as gender and age, influence immune function, particularly IgM production, making this study crucial for understanding their humoral immunity. This study aimed to determine the immunoglobulin M profile of Sumbawa horses raised in the Lar system (a traditional extensive grazing method) of Sumbawa Island.
Materials and methods: Serum samples were collected from 70 Sumbawa horses, categorized by gender (36 males and 34 females) and age group (34 young: below 12 months; 36 adults: above 12 months). The immunoglobulin M levels in the blood serum were measured using the Enzyme-Linked Immunosorbent Assay.
Results: The results indicated detectable immunoglobulin M concentrations in all 70 serum samples, ranging from 1.792 µg/mL to 51.341 µg/mL. The average serum immunoglobulin M levels were higher in female horses (23.8822 ± 12.15941 µg/mL) compared to males (20.1091 ± 9.51380 µg/mL). Similarly, young horses (22.9249 ± 11.90000 µg/mL) exhibited higher immunoglobulin M levels than adults (21.0132 ± 10.08383 µg/mL). Despite these differences, statistical analysis showed no significant differences among the groups.
Conclusion: The average immunoglobulin M levels observed in this study provide baseline data for the immunoglobulin M profile of Sumbawa horses raised under the traditional grazing system on Sumbawa Island, Indonesia.

1. Introduction

Horses hold significant historical, cultural, and economic value in Indonesia. According to the Indonesian Central Statistics Agency[1], the horse population in Indonesia was estimated to reach 367.302 in 2023. West Nusa Tenggara ranks as the third-largest contributor to this population, with 36.900 horses, primarily from Sumbawa Island[2]. The Sumbawa horse, standing 100–127 cm tall, is a distinct breed named after its place of origin, Sumbawa Island[3]. These horses are used for various purposes, including transportation, herding, racing, and wedding dowries. Additionally, their milk, known locally as *kuda liar* milk, is renowned for its health benefits, such as boosting immunity, aiding digestion, promoting skin health, and providing probiotic effects[4].

The majority of regions in West Nusa Tenggara Province, including Sumbawa Island, are predominantly characterized by dry land[5], making it ideal for livestock development, including horse farming. Horses on this island are typically raised in a traditional extensive grazing method, locally referred to as the *Lar system*[6]. The *Lar* System plays a significant role in improving horse productivity due to its efficiency[7]. However, according to Schmitz and Isselstein[8], extensive grazing systems such as the *Lar* system face several challenges, including limited availability of nutritious feed, poor sanitation and hygiene, and inadequate health monitoring. Poor environmental conditions can induce stress, which negatively affects the immune function of horses[9]. One method to evaluate immune function is by measuring antibody (immunoglobulin) production[10]. Among immunoglobulins,

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immunoglobulin M (IgM) is particularly useful for assessing immune function, as its concentration increases significantly during acute infections, making it an indicator of the primary immune response[11]. Several studies have highlighted the importance of Immunoglobulin M (IgM) as an indicator of immune function in horses. Wagner et al.[12] developed monoclonal antibodies specific to IgM for detecting West Nile Virus infections, thereby improving diagnostic sensitivity by monitoring primary IgM responses. Furthermore, studies by Wilkerson et al.[13] and Felippe[14] demonstrated the relevance of IgM in detecting immunodeficiencies and serving as a diagnostic tool in various medical conditions.

The production of IgM as part of the adaptive immune response, is influenced by external factors, such as environmental conditions, and internal factors, such as physiological conditions, including gender and age[15]. According to Giraldo et al.[16], sex hormones, such as estrogen and testosterone can modulate immune responses, suggesting that male and female horses may differ in their ability to combat infections and produce antibodies. Similarly, age influences immune responses, as the immune systems of young animals are still developing and are generally less robust[17]. This study aims to investigate whether significant immunological differences exist between sexes and age groups in the context of immune function. Furthermore, it seeks to contribute to the molecular database on humoral immune function in Sumbawa horses.

2. Material and Methods

2.1 Ethical approval

The Committee of Ethical Clearance of the Faculty of Veterinary Medicine, Udayana University, Denpasar, Indonesia, has approved all of the research activities by providing a certificate of ethical clearance ref. B/211/UN14.2.9/PT.01.04/2024.

2.2 Study area and animal samples

This study was conducted on Sumbawa horses raised under the *lar* system on Sumbawa Island, West Nusa Tenggara Province, Indonesia in August 2024. The location of the study encompassed several villages on Sumbawa Island, including Penyaring, Labuan Sumbawa, Poto, Pungkit, Raberas, Kaking, Semu, Sernu, Brang Biji, Serading, Kerato, Baru Tahan, Berare, and Moyo. A total of 70 clinically healthy horses, confirmed through physical examinations, were grouped based on gender and age including 18 young males, 18 adult males, 16 young females, and 18 adult females. The horses were selected with the assistance of their respective owners, who captured the animals that met the study's criteria within each *lar*. Each village listed above contributed horses from a single farm, resulting in one farm per village being included in the study (Figure 1).

The diet of the horses in this study consisted exclusively of natural forage available around the *lar* system. The primary forage species included *Leucaena leucocephala* (lamtoro), *Cenchrus ciliaris*, *Panicum maximum*, and *Imperata cylindrica*. These plants, along with other leguminous trees such as *Gliricidia sepium* and *Calliandra calothyrsus*, formed the basis of the horses' nutritional intake, reflecting the traditional grazing practices in the region.

Vaccination protocols and antiparasite treatments were not applied to the horses included in this study. According to the owners, no vaccines or antiparasitic drugs were administered, as the traditional management system relies entirely on natural grazing practices and does not include veterinary interventions. This lack of vaccination and antiparasitic treatment aligns with the extensive grazing system in the region, where horses are generally kept under minimal management conditions.

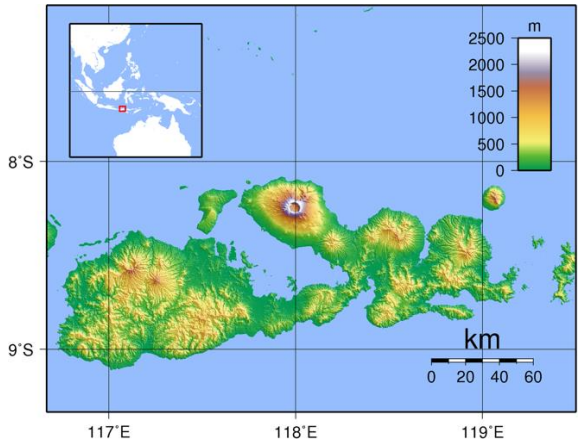

Figure 1. Map of Sumbawa Island, Nusa Tenggara Barat, Indonesia

2.3 Blood sampling

Serum samples were obtained by collecting 10 mL of blood from the jugular vein using a venoject needle. The blood was collected into red-top tubes (*serum separator tubes*, SST), which are specifically designed for serum collection. The serum was allowed to rest at room temperature for approximately 10–20 minutes and then centrifuged at 2000–3000 rpm for about 20 minutes to prevent sediment formation.

2.4 Enzyme-linked immunosorbent assay (ELISA) test

This research was conducted at the Veterinary Immunology Laboratory, Faculty of Veterinary Medicine, Udayana University, Indonesia, in September 2024. The procedure followed a standard ELISA protocol[18] (Wuhan Fine Biotech Co., Ltd., Catalog: EHS0063). A volume of 100 µL of standard solution or sample was added to each well, and the plate was covered and incubated statically for 90 minutes at 37°C. After incubation, the plate was washed twice without soaking. Subsequently, 100 µL of biotin-labeled antibody solution was introduced to each well, followed by covering the plate and incubating statically for 60 minutes at 37°C. The washing process was repeated three times, with a 1-minute soak during each wash. Streptavidin-Biotin Complex (SABC) working solution (100 µL) was then added to each well, and the plate was covered and incubated statically for 30 minutes at 37°C. This was followed by five washes, each with a 1-minute soak. Tetramethylbenzidine (TMB) substrate solution (90 µL) was added to each well, and the plate was covered and incubated statically for 10–20 minutes at 37°C, allowing for accurate Tetramethylbenzidine visualization. Finally, 50 µL of stop solution was applied, and the results were immediately read at a wavelength of 450 nm for calculation.

2.5 Statistical analysis

In this study, the statistical comparisons between the two

groups (age and gender) were conducted using an independent-sample T-test to determine significant differences. Statistical analyses were performed using the Statistical Package for the Social Science (SPSS) software, version 17.0 (SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant at a p-value < 0.05. Values are expressed as the mean ± standard deviation[19].

3. Results

Table 1 presents the comparative data on the mean IgM levels between female and male Sumbawa horses. Sumbawa Island is located in the central region of Indonesia, precisely in the province of West Nusa Tenggara (NTB). The mean IgM level in female horses was 23.88 ± 12.16 µg/mL, while in males, it was 20.11 ± 9.51 µg/mL. Although the IgM levels in female horses were higher than in males, the difference was not statistically significant (Figure 2A, p > 0.05).

The findings presented in Table 2 illustrate the comparison of mean IgM levels between young and adult Sumbawa horses. The data show that the mean IgM level in young horses was 22.92 ± 11.90 µg/mL, while in adult horses, it was 21.01 ± 10.08 µg/mL. Although the IgM level in young Sumbawa horses was higher than in adult horses, the difference was not statistically significant (Figure 2B, p > 0.05).

Table 1. The difference in the average serum Immunoglobulin M (IgM) levels in Sumbawa horses based on gender (µg/mL)

Groups (Gender)	
Male (M ± SD)	**Female (M ± SD)**
20.11 ± 9.51[a]	23.88 ± 12.16[a]

M: Mean; SD: Standard Deviation; a: No significant difference (p > 0.05).

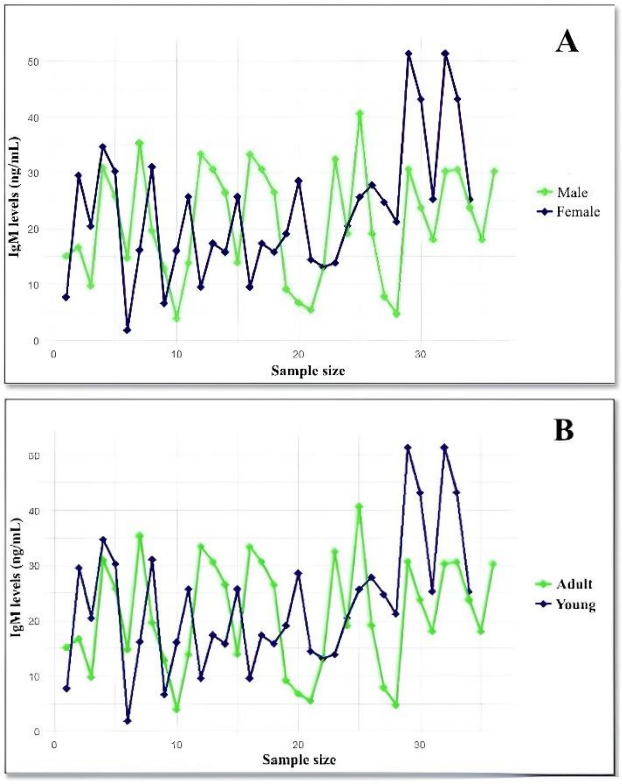

Figure 2. The Immunoglobulin M (IgM) levels in Sumbawa horses raised under the *lar* system on Sumbawa Island were measured and analyzed in this study. A: The comparison of IgM levels based on gender, between male and female horses, B: The comparison of IgM levels based on age, between adult and young horses.

Table 2. The difference in the average serum Immunoglobulin M (IgM) levels in Sumbawa horses based on age (µg/mL)

Groups (Gender)	
Adult (M ± SD)	**Young (M ± SD)**
21.01 ± 10.08[a]	22.92 ± 11.90[a]

M: Mean; SD: Standard Deviation; a: No significant difference (p > 0.05).

4. Discussion

Data on the influence of gender and age on the variation in IgM levels in Sumbawa horses raised under the *lar* system showed no significant differences (p > 0.05). However, the recorded IgM values in this study, approximately 20 mg/dL (200 µg/mL), were notably lower than the values reported in previous studies. According to Perkins et al.[20], IgM concentrations in 103 healthy horses, measured using the radial immunodiffusion assay, ranged from 500 to 2420 µg/mL. Similarly, Horohov et al.[21] reported normal IgM concentrations of approximately 1200 (±310) µg/mL in healthy horses. These discrepancies highlight potential differences in breed-specific immunoglobulin profiles, environmental conditions, or management practices.

The lower IgM levels observed in this study may reflect the unique environmental and physiological factors associated with Sumbawa horses. These horses are raised in tropical climates under the traditional *lar* system, where limited nutritional resources and exposure to environmental stressors are common. Chronic nutritional deficiencies, especially in protein or specific micronutrients critical for antibody synthesis, may suppress IgM production[22]. Additionally, environmental stress, such as high temperatures and parasitic loads, could further impair immune function[23]. The lack of antiparasitic treatment and vaccination in the study population, as reported in the methods, may also exacerbate immune modulation and influence IgM levels.

Although the current study shows that female horses exhibited higher IgM concentrations than males, this difference was not statistically significant. This trend aligns with broader immunological findings across species, where females generally demonstrate stronger humoral immune responses. For instance, Fischinger et al.[24] reported that females tend to exhibit higher immunoglobulin levels due to hormonal modulation, particularly estrogen, which enhances antibody production and immune cell activation. Similarly, Giraldo et al.[25] highlighted that sex hormones, such as estrogen and testosterone, influence immune responses, potentially explaining the observed variations between male and female horses.

Additionally, Uner et al.[26] observed that female horses had larger monocyte counts, which could enhance phagocytic activity and cytokine production, indirectly contributing to higher antibody levels. While these findings provide a biological basis for differences in IgM levels between sexes, the current study's results must be interpreted cautiously due to the lack of statistical significance and the limited sample size. Further research with larger, more diverse populations is necessary to establish whether this trend is consistent across different equine breeds and environmental conditions.

The higher IgM levels observed in young Sumbawa horses compared to adults may be attributed to developmental immunological demands and the unique environmental conditions of the *lar* system. This traditional

grazing system, characterized by natural forage and minimal veterinary intervention, exposes horses to diverse antigens from plants such as *Leucaena leucocephala* and *Imperata cylindrica*, potentially driving immune activation. Similar to findings by Sturgill et al.[27], young horses rely heavily on IgM for early immune protection against these antigens, as their adaptive immunity is still maturing. The absence of vaccination and antiparasitic protocols in this study further underscores the role of natural antigenic exposure in modulating immune responses. Additionally, the high antigenic pressure and environmental stressors, such as parasitic loads and tropical climatic conditions, may enhance IgM synthesis in foals, aligning with observations by Zandoná Meleiro et al.[28]. However, the limited nutritional resources inherent in the *lar* system may simultaneously restrict immune efficiency, as protein and micronutrients like zinc and selenium—critical for antibody synthesis—are often deficient[22]. The breed-specific immunological adaptations of Sumbawa horses, adapted to these harsh conditions, likely play a role, as Horohov et al.[21] noted that environmental and genetic factors significantly influence immune profiles in horses. Despite these insights, the relatively small sample size (70 horses) and confinement to one geographic region limit the generalizability of these findings. Future studies incorporating larger populations and diverse management systems are necessary to further elucidate the dynamics of IgM production in young and adult horses.

5. Conclusion

A total of 70 samples were analyzed, revealing varying Immunoglobulin M (IgM) levels ranging from 1.79 µg/mL to 51.34 µg/mL. While the serum IgM levels in female Sumbawa horses (23.88 ± 12.16 µg/mL) were higher than those in males (20.11 ± 9.51 µg/mL), and IgM levels in young horses (22.92 ± 11.90 µg/mL) exceeded those in adults (21.01 ± 10.08 µg/mL), these differences were not statistically significant. The findings suggest potential trends in IgM variation influenced by gender and age; however, the limited sample size, the focus on a single geographic region, and the reliance on horses managed under the *lar* system restrict the generalizability of the results. Future studies should incorporate larger, more diverse sample populations and include comparisons with horses raised in different environments and management systems to provide a broader understanding of IgM dynamics in Sumbawa and other equine breeds.

Declarations

Competing interests

The author declared that there are no conflicts of interest.

Authors' contributions

I Made Bagi Rate, Ni Ketut Suwiti, and I Nengah Kerta Besung designed the research, finalized the manuscript, and collected the data, Ida Bagus Kade Suardana analyzed the data and finalized the manuscript. All authors reviewed and confirmed the final manuscript.

Authors' relationships and activities

All authors disclose any personal and financial relationships with other people or organizations.

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Availability of data and materials

The author will provide all necessary data to the editor upon request.

Ethical considerations

All authors have reviewed the manuscripts for ethical concerns, such as plagiarism, consent to publish, misconduct, data fabrication and falsification, double publishing and submission, and redundancy.

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